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STUDIES OF PHLEBOTOMINE SAND FLIES

FINAL REPORT

by

David G. Young

June 1982

Supported by

**U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012**

Contract No. DADA17-72-C-2139

University of Florida
Gainesville, Florida 32611

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The phlebotomine sand fly collection grew during this contractual period from 197 American species and subspecies to 282 taxa, making it the most complete reference collection of New World species in existence. A total of 66 holotypes are housed in this collection. Descriptions of 23 species discovered during the past ten years were published; at least 10 other undescribed species were also discovered. Identification keys to the subfamilies of psychodidae, the genera of phlebotominae, the subgenera of Lutzomyia, were prepared. Cer-		

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certain subgenera of Lutzomyia were reviewed; a large work on the phlebotomines of Colombia, representing nearly a third of the New World species, was published. Additional illustrated keys were completed for the species of Kenya and for certain species groups of Lutzomyia. Significant progress was made on a handbook of the neotropical species and reviews of the sand fly fauna of Guatemala, Ecuador and North America were written.

A paper on the classification of Phlebotominae was published. It now serves as a basis of classification for the subfamily and is being used by most investigators.

→ New techniques, developed for rearing and colonizing phlebotomines in our laboratory, resulted in the successful laboratory colonization of nine out of nine species collected. Eight of these species, including Phlebotomus martini - the vector of visceral leishmaniasis in Kenya, were colonized for the first time. The first demonstration of vertical transmission of a Phlebovirus in one colonized species was viriologically confirmed for the first time. Leishmania was successfully transmitted to hamsters by the bites of lab-bred Lutzomyia anthophora, L. shannoni and L. diabolica. No information on their vector potential was previously known.

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FINAL REPORT

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SUMMARY

The phlebotomine sand fly collection grew during this contractual period from 197 American species and subspecies to 282 taxa, making it the most complete reference collection of New World species in existence. A total of 66 holotypes are housed in this collection. Descriptions of 23 species discovered during the past ten years were published; at least 10 other undescribed species were also discovered. Identification keys to the subfamilies of Psychodidae, the genera of Phlebotominae, the subgenera of Lutzomyia, were prepared. Certain subgenera of Lutzomyia were reviewed; a large work on the phlebotomines of Colombia, representing nearly a third of the New World species, was published. Additional illustrated keys were completed for the species of Kenya and for certain species groups of Lutzomyia. Significant progress was made on a handbook of the neotropical species and reviews of the sand fly fauna of Guatemala, Ecuador and North America were written.

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Final Report
DADA 17-72-C-2139

A. Introduction

This report covers the period from 1 June 1972 to 30 June 1982. The major objectives of the contract were to build an American reference collection of phlebotomine sand flies, to prepare keys, illustrations and other aids to identification and to arrive at a more satisfactory classification of the subfamily. The difficulties of establishing and maintaining laboratory colonies of these insects have been recognized for many years and some effort was also made during this time period to develop techniques for rearing phlebotomines in the laboratory.

Following the U.S. Medical Research & Development Command guidelines, this final report "shall be a narrative summary covering research performed during the entire contract period."

B. Accomplishments

1. Development of a phlebotomine reference collection

At the beginning of the contract period, 305 species and subspecies of American Phlebotomine were known in the genera Lutzomyia (278 spp.), Brumptomyia (23 spp.), Warileya (3 spp.) and Hertigi (1 sp.). Our collection, at that time, was represented by 197 species in these genera.

As of June, 1982, the American Phlebotominae fauna consisted of 363 species and subspecies, the majority of the species occurring in South America. Our reference collection had also grown with 282 species represented, making it the most complete reference collection of American Phlebotominae in the world. For future studies on the taxonomy of the group, this collection will be an indispensable re-

source. Over 100 species of Old World species are also contained in the collection.

Material was obtained from colleagues working in nearly all New World countries and from contract-supported trips made by the Principal Investigator. Countries visited included Colombia (1973, 1975), Brazil (1972, 1974, 1979), Ecuador (1976), Dominican Republic (1981, 1982) and Kenya (1981). Significant collections were sent to the PI from colleagues in Mexico, Guatemala, Colombia, Brazil, Trinidad, Surinam, Bolivia and Ethiopia.

From these collections, 23 species were described as new, 10 others are awaiting description from the following countries - Mexico, Guatemala, Costa Rica, French Guiana, Ecuador, Peru and Brazil. At least 7 of these recently described or undescribed phlebotomines are anthropophilic and one, Lutzomyia yuilli, originally described from Colombia, was later found naturally infected with Leishmania in Western Brazil.

Smaller lots of specimens were given to the PI by workers in El Salvador, Honduras, U.S.A., Panama and French Guiana. These, in addition to the other material, helped determine the overall geographic ranges of some species and provided material for the study of intraspecific variation and classification at the subgeneric level. All specimens are preserved on microslides or in alcohol following maceration.

It is clear that the most significant accomplishment achieved under this contract was the development of an outstanding and unique reference collection of American Phlebotominae. Holotypes of 66 species and paratypes of 88 species are represented. All holotypes, except for L. olmeca nociva from Brazil, will eventually be deposited in the U.S. National Museum. A list of the holotypes is given in Appendix I. A

list of all the New World species represented in the collection will be given in the Annual Report, 1 Feb. 1983, under DAMD 17-82-C-2223.

2. Preparation of keys, illustrations and other aids for identifying sand flies

Beginning with the 1974 Annual Report under this contract, illustrated identification keys to the subfamilies of Psychodidae, genera of Phlebotominae and subgenera of Lutzomyia were provided. This report was widely circulated to colleagues and has been cited numerous times. The published paper on the "Bloodsucking psychodid flies of Colombia" repeats the keys to the subfamilies of Psychodidae and has additional keys to the New World genera, the subgenera of Lutzomyia and the species of Colombia which represent over one fourth of the described American species. Regional keys to the species of Lutzomyia were prepared for Panama, Trinidad and North America, the fauna of the latter region being treated in detail in a nearly completed paper given in the Appendix of the 1982 Annual Report.

Working keys to the sand flies of Kenya were completed in 1980 to help WRAIR investigators identify specimens encountered during field work on leishmaniasis. A four-week trip to that country in 1981 by the PI resulted in the discovery of Phlebotomus duboscqi, an important vector of cutaneous leishmaniasis in Africa, that had not been previously reported from Kenya. Papers reporting this finding and the discovery and description of the male of Sergentomyia blossi, previously unknown, were later published.

Illustrations of about 160 American species were completed by the PI for inclusion in a handbook covering the neotropical region species. Some shorter papers dealing with identification of species groups and their taxonomy were published or were completed during this contract period (eg., review of the subgenus Evandromyia). Keys

to other subgenera of Lutzomyia (eg. Dampfomyia, Lutzomyia, Viannamyia, longispina group, dreisbachi group, baiti group and Psychodopygus) were included in the Annual Reports of 1975, 1976.

Prepared by Dr. G.B. Fairchild (Co-principal Investigator on this contract from 1972-1975), identification keys to sand flies of Panama were to be included in a large paper on the ecology of leishmaniasis in Panama. It was later decided to exclude these keys because those available for the Colombian species, and already published, applied to the Panamanian fauna as well. Dr. Fairchild, who received support from this contract while working on part of this paper, is the second author. The manuscript is now in press (J. Med. Entomol.).

A preliminary report on the sand flies of Guatemala, including 25 species, was given in the 1981 Annual Report. One species is undescribed; 13 others represent new records for the country. Since that report, 10 additional species have been found by Dr. C.H. Porter who is collaborating with the PI on this study. A detailed paper on the Phlebotomine fauna of Guatemala, is in preparation.

A review of the Microps group species with illustrations of each species (Annual Report 1981) was completed but the discovery of two additional species in the group in 1982 from South America has delayed its publication. Similarly, new collections of sand flies from Ecuador have increased the known fauna there and a review of these species will be completed in 1983. Substantial progress was made on the handbook of the neotropical phlebotomies but the task proved greater than originally thought and it will not be finished until 1984.

3. Classification of the Phlebotominae

A proposed classification of this subfamily was published in 1977 by D.J. Lewis, D.G. Young, G.B. Fairchild and D.M. Minter and has been widely accepted. The genus Hertigia is considered to be a junior synonym of Warileya, a small genus of 5 species. Details regarding subgenera of Lutzomyia, phlebotomus and Sergentomyia were given in that paper and will not be repeated here.

4. Other activities and accomplishments

Rearing techniques for the laboratory colonization of phlebotomines were developed during this time period. A standard larval diet proved successful in the rearing of 9 species including Phlebotomus martini, a vector of visceral leishmaniasis in Kenya, that the PI helped colonize in the laboratory for the first time. The establishment of this colony, and others, has provided, for the first time, sufficient specimens of known age for experimental work on phlebotomine-borne diseases.

The first demonstration of transovarial transmission of a Phlebovirus, Rio Grande virus, by sand flies (Lutzomyia anthophora) was shown using lab-bred specimens. The experimental work was done by R.G. Endris working with the PI and Dr. R. Tesh with partial support from this contract. Data on the biology of this sand fly species were compiled and the completed paper was sent to the J. Med. Entomol. for publication.

Leishmaniasis transmission by bite was demonstrated using lab-bred L. shannoni, L. anthophora and L. diabolica. These sand flies were allowed to feed on Leishmania mexicana-infected hamsters, then after oviposition, the surviving females were exposed to a noninfected hamster. Each of the species transmitted the disease under laboratory conditions. No information on their vector potential had

been known previously.

The rearing technique, now being used in several laboratories throughout the world, will also provide material for studying the taxonomy of sibling species (eg., the vector group of the sub-genus Synphlebotomus in Africa) and for studying the immature stages, a neglected area of taxonomic research. A descriptive paper on the surface structure of eggs of 5 Lutzomyia species, reared in our laboratory, was given in the 1982 Annual Report and will be submitted for publication in 1983.

A review of the pathogens known to infect phlebotomines resulted in the publication of two papers during this time period.

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APPENDIX I

List of Phlebotominae holotypes in the University of Florida collection.

Genus Warileya

1. W. hertigi ♂ Panama
2. W. nigrosacculus ♀ Panama
3. W. phlebotomanica ♂ Peru
4. W. rotundipennis ♂ Panama

Genus Brumptomyia

5. B. galindoi ♂ Panama
6. B. hamata ♂ Panama

Genus Lutzomyia

(Subgenus Lutzomyia)

7. L. marinkeleii ♂ Colombia
8. L. vexillaria ♂ Panama

(Verrucarum Group)

9. L. christophei ♂ Dominican Republic
10. L. moralesi ♂ Colombia
11. L. odax ♂ Panama
12. L. oresbia ♂ Panama
13. L. orestes ♂ Cuba

(Vespertilionis Group)

14. L. aquilonia ♂ U.S.A.
15. L. deleoni ♂ Guatemala
16. L. steatopyga ♂ Mexico
17. L. vesicifera ♂ Panama
18. L. viriosa ♂ Panama

(Delpozoi Group)

19. L. inusitata ♂ Mexico

(Subgenus Dampfomyia)

20. L. insolita ♂ Panama
21. L. permira ♂ Mexico
22. L. rosabali ♂ Panama
23. L. rubidula ♀ Panama

(Subgenus Pressatia)

24. L. acanthobasis ♂ Panama
25. L. dysponeta ♂ Panama

(Baityi Group)

26. L. hansonii ♂ Panama

(Migonei Group)

27. L. xerophila ♂ U.S.A.

(Shannoni Group)

28. L. cratifer ♂ Mexico

29. L. dasymera ♂ Panama

30. L. soccula ♂ Panama

31. L. undulata ♂ Guatemala

32. L. volcanensis ♂ Panama

(Longispina Group)

33. L. triramula ♂ Panama

(Aragaoi Group)

34. L. barrettoi majuscula ♂ Panama

35. L. carpenteri ♂ Panama

36. L. runoides ♂ Panama

37. L. aclydifera ♂ Panama
(Subgenus Trichophoromyia)

38. L. cellulana ♂ Colombia

39. L. howardi ♂ Colombia

40. L. reburra ♂ Panama

41. L. saltuosa ♂ Colombia
(Subgenus Nyssomyia)

42. L. edentula ♀ Guatemala

43. L. olmeca bicolor ♂ Panama

44. L. olmeca nociva ♂ Brazil

45. L. trapidui ♂ Panama

46. L. ylephiletor ♂ Panama
(Subgenus Psychodopygas)

47. L. bispinosa ♂ Panama

48. L. carrerai thula ♂ Panama

49. L. nicaraguensis ♂ Nicaragua

50. L. fairtigi ♂ Colombia
(Vexator Group)

51. L. cirrita ♂ Colombia

52. L. hartmanni ♂ Panama

53. L. sanguinaria ♂ Panama

54. L. strictivilla ♂ Colombia

55. L. vargasi ♂ Mexico
(Cayennensis Group)

56. L. californica ♂ U.S.A.

57. L. cayennensis hispaniolae ♀
Dominican Republic

58. L. c. jamaicensis ♀ Jamaica

59. L. c. maciasi ♀ Mexico

60. L. c. puertoricensis ♀
Puerto Rico

61. L. c. viequensis ♀ Puerto Rico

62. L. ctenidophora ♀ Mexico

63. L. cubensis ♀ Cuba

64. L. duppyorum ♀ Jamaica
(Ungrouped)

65. L. ignacaci ♂ Venezuela

66. L. pia ♂ Panama

Personnel Supported on Project

G.B. Fairchild, Ph.D. (1972-1976), Professor, Dept. of Entomology & Nematology, University of Florida.

D.G. Young, Ph.D. (1972-1982), Associate Research Scientist, Dept. of Entomology and Nematology, University of Florida.

S.S. Haney (1972-1982), Technician, Dept. of Entomology and Nematology, University of Florida.

Dr. R.G. Endris and Dr. P.V. Perkins were graduated in 1982 with Ph.D. degrees. Both received partial support (no salaries) from this contract in the form of equipment used.

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